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The social evolution of dispersal with public goods cooperation

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Abstract

Selection can favour the evolution of individually costly dispersal if this alleviates competition between relatives. However, conditions that favour altruistic dispersal also mediate selection for other social behaviours, such as public goods cooperation, which in turn is likely to mediate dispersal evolution. Here, we investigate – both experimentally (using bacteria) and theoretically – how social habitat heterogeneity (i.e. the distribution of public goods cooperators and cheats) affects the evolution of dispersal. In addition to recovering the well-known theoretical result that the optimal level of dispersal increases with genetic relatedness of patch mates, we find both mathematically and experimentally that dispersal is always favoured when average patch occupancy is low, but when average patch occupancy is high, the presence of public goods cheats greatly alters selection for dispersal. Specifically, when public goods cheats are localized to the home patch, higher dispersal rates are favoured, but when cheats are present throughout available patches, lower dispersal rates are favoured. These results highlight the importance of other social traits in driving dispersal evolution.

Introduction

Understanding dispersal is a major aim of evolutionary ecology (Weins, 2001). Theoretical work suggests that dispersal provides possible benefits when the environment varies in time (Van Valen, 1971; McPeck & Holt, 1992) and if it reduces inbreeding depression (Bengtsson, 1978) and kin competition (Hamilton & May, 1977). Conversely, dispersal can be opposed by obvious costs, such as increased mortality or reduced reproduction (Rousset & Gandon, 2002; Bonte *et al.*, 2012). The indirect fitness benefits of dispersal, that arise owing to it alleviating kin competition, highlight that dispersal is often a social trait and that it can be favoured by kin selection even in the context of severe fitness penalties for the dispersing individuals (Hamilton & May, 1977; Comins *et al.*, 1980; Taylor & Frank, 1996; Gandon & Michalakis, 1999; Taylor & Buckling, 2010; Wei *et al.*,

2011). The evolution of altruistic traits often requires high relatedness, and in terms of dispersal, this would mean a tendency for individuals who share the same ‘dispersal alleles’ to be associated in space. However, dispersal itself is likely to reduce relatedness, which in turn can reduce selection for dispersal (Taylor, 1988; Gandon, 1999; Gandon & Michalakis, 1999; Ronce, 2007).

The change in population structure resulting from dispersal is likely to have particularly important consequences for the evolution of dispersal rates when other social traits have important fitness consequences (Perrin & Lehmann, 2001 and references therein; Le Galliard *et al.*, 2005). High relatedness, as well as selecting for elevated dispersal rates, also selects for other forms of altruism, where individuals pay a cost for the benefit of the group as a whole (Hamilton, 1964). In contrast to dispersal, where leaving the group is often an altruistic act, many altruistic traits require individuals to stay in groups. This has led to the development of theoretical models that address coevolution between social behaviours and dispersal behaviour (Koella, 2000; Perrin & Lehmann, 2001; Le Galliard *et al.*, 2005). A

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key prediction from these models is that selection for dispersal will be reduced when other cooperative behaviours have important direct or indirect fitness benefits.

We investigate how selection for dispersal in bacteria is influenced when individuals are cooperating (or not) by the production of public goods. Wild-type bacteria produce numerous extracellular molecules, such as tissue degrading enzymes, iron-scavenging siderophores and sticky polymers, to protect surface-growing bacteria (biofilms), which are individually costly but benefit the group as a whole (West *et al.*, 2007). Such behaviours are readily exploitable by nonproducing cheats; hence, dispersal might be selected against in public good producing bacteria if it increases the chance of encountering exploiting cheats. Conversely, selection for dispersal may be beneficial if it allows escape from social exploitation or if it promotes individuals to move from saturated to empty patches. We investigate how the evolution of dispersal is affected by public goods production and exploitation when exploiters are locally (i.e. exhibit low patch occupancy) or widely (i.e. exhibit high patch occupancy) distributed. We define patch occupancy as the fraction of patches that are occupied. We explore this result experimentally and theoretically, with the aim to link empirical data with general theory of the evolution of dispersal.

We use a well-studied bacteria model for social evolution, the opportunistic bacterial pathogen, *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* possesses a range of motility mechanisms, which trade-off against each other, to move in different environments (Bardy *et al.*, 2003; Taylor & Buckling, 2011). We manipulate dispersal behaviour using two genetically modified pili mutants of *P. aeruginosa*, which in a semi-solid agar plate show disparate dispersal behaviours: one acts as a 'disperser' and is able to quickly colonize the plate due to the ability to move freely through the substrate; the other acts as a 'nondisperser' and is unable to colonize the plate as quickly due to restricted movement (details of genotypes are given in Materials and methods). Consistent with theoretical results, our previous work using these mutants has shown that conditions of high relatedness favour the disperser (Taylor & Buckling, 2010).

Pseudomonas aeruginosa produces numerous public goods but, for simplicity, we focus upon a single trait: extracellular iron-chelating siderophores (Ratledge & Dover, 2000; West & Buckling, 2003). Iron is vital for bacterial growth; however, most iron in the natural environment exists in the insoluble ferric form and must be reduced via reactions initiated by siderophore molecules to be utilized. Under conditions of iron limitation, clonal populations of siderophore producers reach much higher densities (and lead to more severe infections) than isogenic mutants that do not produce the primary siderophore, pyoverdine (West & Buckling,

2003). However, nonproducing mutants can exploit the pyoverdine of producers and hence outcompete them, when in direct competition as a result of the metabolic cost of pyoverdine production (Griffin *et al.*, 2004).

Materials and methods

Strain details and growth conditions

Two transposon mutants defective in type IV pili and generated from a wild-type strain of *P. aeruginosa* (PAO1) were used as dispersing phenotypes: PilA acts as the disperser as it is absent of pili (surface organelles which aid motility on hard surfaces), and PilU acts as the nondisperser as it is able to express but unable to retract pili (hyperpiliated) (D'Argenio *et al.*, 2001). These mutants are isogenic, and hence, siderophore production does not differ between strains. A soft agar medium creates conditions whereby PilA is able to move freely through the substrate, whereas the drag caused by permanently extruded pili causes PilU to become stuck. A third mutant defective in siderophore production (PAO1 Δ pvdD *pchEF*; Ghysels *et al.*, 2004) was used as the 'cheat'. This strain is unable to grow in isolation in an iron-limited environment and requires access to a siderophore-producing strain to grow. The dispersing phenotype of the cheat is intermediate between PilA and PilU; therefore, PilA is able to disperse beyond the range of cheats, but PilU is not. For comparison, a wild-type PAO1 strain was used as the 'cooperator' (its dispersing phenotype is equivalent to the cheats, but they produce siderophores and therefore do not impose a social cost). Bacterial cultures were grown overnight at 37 °C in 6 mL Casamino acid media (CAA) shaken at 0.9 g. Cells were then pelleted and resuspended in M9 buffer solution twice to ensure that all nutrients were removed from the media.

Any free iron which potentially remained in resuspended cultures was removed by the addition of an iron chelator (100 μ g mL⁻¹ of human apo-transferrin and 20 mM NaHCO₃; Sigma; Meyer *et al.*, 1996; Griffin *et al.*, 2004). Bacteria were left to grow and disperse for 72 h.

Treatment conditions

Our simple factorial experimental design involved determining the fitness (growth) of cooperating dispersers (PilA) and nondispersers (PilU) in the presence of one of two competitors – cheats (exploiter) or cooperators (WT) – which were distributed throughout the plate (high-occupancy treatment) or only present in the inoculation site (low-occupancy treatment). This resulted in eight experimental treatments (Fig. 1). A total of 25 mL of iron-limited CAA agar (0.6% w/v agar) was poured into 20-cm-diameter Petri dishes and allowed to dry in the laminar flow hood for 20 min.

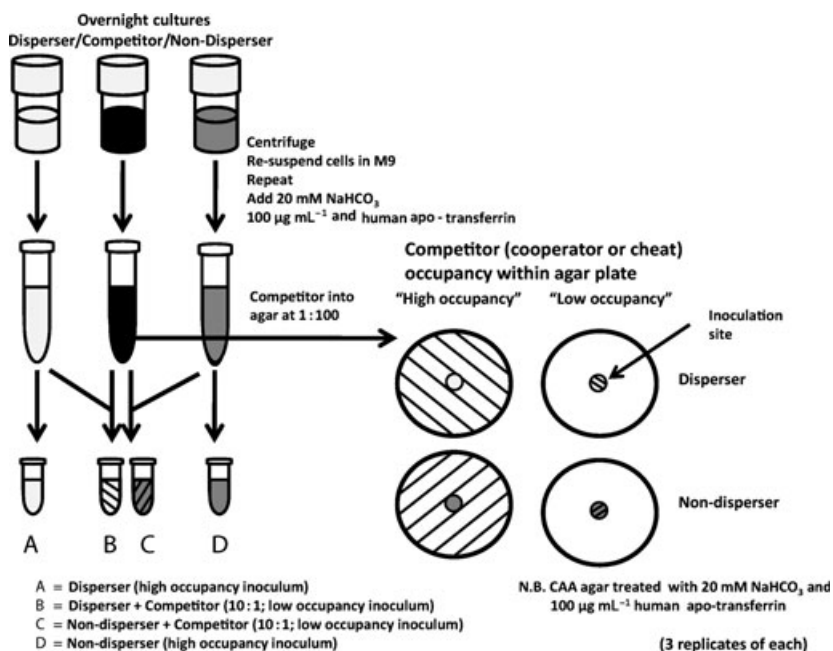


Fig. 1 Methods for setting up the eight treatment groups: competition is either low or high occupancy; the competitor is either a cooperator or a cheat; and the dispersing variant is either a disperser (PilA) or a nondisperser (PilU). Under the high-occupancy treatment, agar is supplemented with a competitor strain (either a cooperator or cheat), which will compete for space and resources. The disperser or nondisperser is inoculated into the centre of each plate. Under high-occupancy treatment, this inoculum is pure, but under low-occupancy treatment, this inoculum is mixed with one of the competitor strains at a concentration of 10 : 1.

Agar plates used for high-occupancy treatment were supplemented with 250 µL (approximately 10^9 cells) overnight competitor culture (either cheat or cooperator), and the other half remained bacteria free. Inoculum was prepared as follows: (i) low-occupancy treatment: competitors (cheats or cooperators) were mixed in an Eppendorf with each of the dispersal variants (disperser or nondisperser) at 1 : 10; (ii) high-occupancy treatment: pure disperser (PilA) and nondisperser (PilU) cultures were used as the inoculum, and 2.5 µL of inoculum (approximately 10^7 cells) was pipetted into the centre of the agar plate of the corresponding treatment group (i.e. low-occupancy or high-occupancy treatment). Each treatment was replicated three times.

It was necessary to address whether pleiotropic effects from the deleted PilA and PilU genes could lead to one of the dispersing variants being an intrinsically worse competitor than the other, due to costs incurred from pili production (or lack of). Cooperator and cheat strains were acting as environmental factors to dispersing strains, and their competitive behaviour was not the focus of this study, but is considered elsewhere (e.g. Jiricny *et al.*, 2010; Kümmerli *et al.*, 2009a). We compared growth rates (to test the relative fitness of the disperser (PilA) with the nondisperser (PilU)) under conditions where motility would confer little or no advantage. We grew the dispersal variants together (approximately 1.2×10^7 cells of disperser and nondisperser) at 37 °C in 6 mL liquid KB shaken at 0.9 g and found growth rates to be equivalent (Wilcoxon, $P = 0.993$). In addition, we also competed the genotypes where bacteria were evenly inoculated through-

out soft agar; hence, moving from one colonized 'patch' would simply result in entering another. Again, we found no significant difference in relative fitness between the two strains (Wilcoxon, $P = 0.14$).

Data collection

Methods were as in Taylor & Buckling (2010). Samples were taken using a 1-mL pipette (Finn pipette), at regular 5-mm intervals along the radius of the colony. The samples were then washed in M9 buffer ($12.8 \text{ g L}^{-1} \text{ Na}_2\text{HPO}_4$, $3 \text{ g L}^{-1} \text{ KH}_2\text{PO}_4$, $0.5 \text{ g L}^{-1} \text{ NaCl}$, $1 \text{ g L}^{-1} \text{ NH}_4\text{Cl}$), diluted to an appropriate dilution to allow colony differentiation and plated to count colony-forming units (CFUs). The relative fitness is always measured between dispersers (PilA) and nondispersers (PilU), and determined by calculating the ratio of the total number of each cell type across corresponding plates. The nature of the experimental design means that in some treatments, there are competitors present, and in others, they are not. However, the fully factorial design of the experiment means that all combinations are comparable.

Visual differentiation was made between siderophore-producing (green) and nonproducing colonies (white) on KB agar. In plates where all strains were cooperators, differentiation could be made between dispersal variants by the colony morphology: on hard agar, the wild-type cooperator (which has fully functional pili) can move more efficiently than the dispersal variants and therefore produces larger colonies than the dispersing or nondispersing phenotypes. The relative fitness of dispersers (PilA) vs. nondispersers (PilU) was

determined by dividing the cell density of dispersers by the cell density of nondispersers across randomly paired plates within the same treatment group (for example: disperser vs. nondisperser under treatment, competitor = cheat; competitor distribution = high occupancy). All analyses and figures were produced on PASW Statistics 18 (SPSS; part of IBM UK Ltd, Middlesex, UK).

Theoretical model

To link the empirical data with the existing general theory of dispersal evolution, we developed a theoretical model to investigate the dynamics between public goods cooperation and kin competition in the context of evolving dispersal behaviour. Our model is relatively simple, as we are mostly interested in qualitative predictions, but it nevertheless captures the main aspects of our experimental design and enables generalization to other populations. We derived an expression for the personal fitness of a cooperator exhibiting a rare genetic variant dispersal strategy. We assume that only cooperators are able to disperse (with varying probability), and therefore, the genetic variation for dispersal is only in cooperators. Thus, this genetic variation at the dispersal locus does not correlate with genetic variation at the cooperation locus (because cooperation is constant among cooperators). We then employed a neighbour-modulated fitness approach to kin selection analysis (Taylor, 1996; Taylor & Frank, 1996; Frank, 1997, 1998; Rousset, 2004; Taylor *et al.*, 2007) to identify the evolutionary equilibrium rate of dispersal, which we then checked for convergence stability (Eshel, 1983; Taylor, 1996).

We assume an infinite metapopulation with three types of patches that vary in their quality (where patch quality is defined by their carrying capacity). Each patch type occurs at a given fixed frequency in the population, which is independent of trait value. A proportion α of patches can contain both cooperator and cheat bacterial cells ('occupied patches'), a proportion β of patches are empty ('vacant patches') and a proportion $\gamma = 1 - (\alpha + \beta)$ of patches contain only cheats ('cheat patches'). Under this patch arrangement, the only way to escape social cheats is by dispersing to another patch. As in the experimental design, we assume that these quantities are fixed parameters.

We denote the genetic relatedness (with respect to the genes for dispersal) among cooperators within patches by r . Each cooperator disperses with independent probability z to a random patch or else remains in their natal patch with probability $1 - z$. Following dispersal, bacteria reproduce asexually, with cooperators reaching carrying capacities of aK in occupied patches, $(1 - a)K$ in vacant patches and 0 in cheat patches. Thus, by varying the value of the parameter a between 0 and 1, we are able to investigate the whole range of possibilities for the impact of patch occupancy upon carrying

capacity, from occupied patches having vastly lower carrying capacity ($a \rightarrow 0$) to vastly greater carrying capacity ($a \rightarrow 1$), than vacant patches. The parameter K acts as a scaling factor, to reflect that there may be numerous bacterial cells in a patch. Note, that the carrying capacity aK includes the cooperators already present in the patch and any new migrants that enter. This parameter defines the carrying capacity of the focal strain only, rather than that of the total bacterial population. That is, we allow for occupancy (relative to vacancy) to either deteriorate a patch, resulting in a decreased carrying capacity ($a < 1/2$), or improve a patch, resulting in an increased carrying capacity ($a > 1/2$). A simple biological interpretation of this difference is that social cheats reduce carrying capacity in the former, and the presence of public goods cooperators increases carrying capacity in the latter (Griffin *et al.*, 2004). For example, (i) migration to empty patches might have a phenotypic effect on cooperators that reduce their ability to cooperate (as in the 'benefits of philopatry' hypothesis; Stacey & Ligon, 1987, 1991), or (ii) empty patches may be intrinsically inferior habitats (as in the 'habitat saturation' hypothesis; Emlen, 1982). In other words, cheats effectively act as an environmental hazard which will reduce the fitness of cooperators who share their space (cf. Frank, 2010) – the relative density of cooperators to cheats will determine carrying capacity of the patch and as such the fitness cost imposed. We assume that cheats do not disperse because further growth is impossible for a cooperator in a pure cheat patch, and cheats are unable to survive in the absence of cooperators. A key assumption in this model is that cheats do not disperse, whereas cheats within the experimental setting exhibit intermediate dispersal. However, the important factor is that dispersers are able to escape cheats, whereas nondispersers cannot, and this was also the case in the experiments. Cooperators can occupy a patch by themselves if they disperse to empty patches, and when $a = 0.5$ and $\gamma = 0$, then our model behaves as if all patches were cooperators only. Given these assumptions, cooperators can only exhibit meaningful growth in initially occupied patches and in vacant patches. Taken together, these two types of patches make up a fraction $\alpha + \beta$ of the total population. Thus, it is convenient to define the proportion of habitable patches that are occupied as $p = \alpha / (\alpha + \beta)$. Full details are given in the Data S1.

Results

Experimental

We measured the relative fitness of the dispersers (Pila) compared with the nondispersers (PilU) for each of the eight treatments (resulting in four average relative fitness values, because each comparable treatment for

dispersers and nondispersers would be randomly paired, and the relative fitness value between the treatments calculated) (Fig. 2). The effect of high-occupancy vs. low-occupancy conditions on the relative fitness of the disperser depended on whether the dispersers were competing with cheats or cooperators (Two-way ANOVA, interaction between Treatment*Competitor; $F_{1,8} = 17.215$; $P = 0.003$). Specifically, it is better to disperse when cheats occupy patches at a low rate (One-sample t -test, test value = 1: $t_2 = 14.28$; $P = 0.01$), but better to remain sessile when cheats are at high occupancy and distributed throughout the environment (One-sample t -test, test value = 1: $t_2 = 374.71$; $P < 0.001$). By contrast, the disperser maintains a fitness advantage across both treatment groups in the presence of cooperators (One-sample t -test, test value = 1: low occupancy, $t_2 = 7.79$; $P = 0.032$; high occupancy, $t_2 = 41.42$; $P = 0.002$).

Theoretical

We use our model assumptions to determine the convergence stable (CS; Eshel & Motro, 1981; Eshel, 1983; Christiansen, 1991; Taylor, 1996) dispersal strategy, z^* (see Theoretical model, Materials and methods and Data S1). An important special case of our model is where we assume the absence of vacant patches ($\beta = 0$, and hence

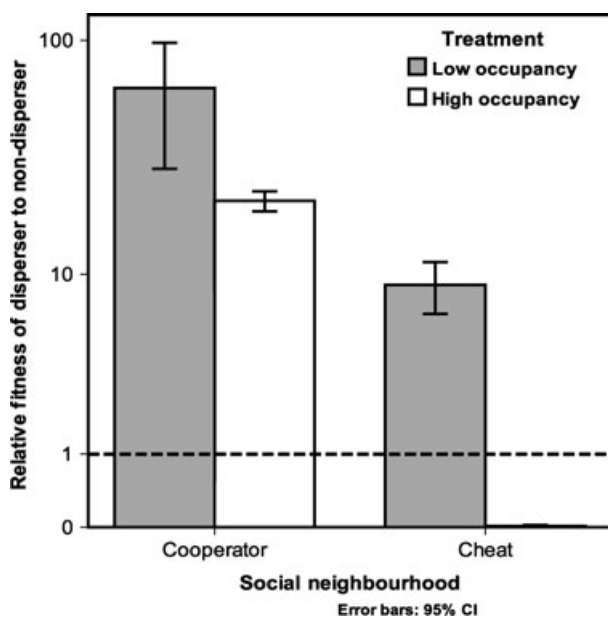


Fig. 2 Average fitness of dispersers (PilA) relative to nondispersers (PilU) in environments which vary in competitor type which form the social neighbourhood (dispersal variants are competing with either cooperators or cheats), and the structure of the social neighbourhood, with competitors distributed throughout the agar (high occupancy) or located only within the colonization patch (low occupancy). Error bars represent \pm 95% CI, and the dashed line indicates a relative fitness of 1, that is, when the fitness of the disperser and the nondisperser are equivalent.

$p = 1$) and clonal relatedness among the cooperators in each patch ($r = 1$): this is equivalent to the model of Hamilton & May (1977), and here, we recover their key result, $z^* = 1/(1 + c)$, where the cost of dispersal (c) is simply the probability of landing upon a cheat patch (i.e. $c = \gamma$). In this classic model, increasingly costly dispersal favours a lower rate of dispersal. However, a surprisingly high rate of dispersal is nonetheless favoured despite even extreme costs (e.g. $z^* \rightarrow 1/2$ as $c \rightarrow 1$).

More generally, analysis of our model reveals that relatedness (r), costs of dispersal ($c = \gamma$), the proportion of habitable patches that are occupied ($p = \alpha/(\alpha + \beta)$) and the relative carrying capacity of occupied patches (a) can interact to mediate the evolution of dispersal. Increasing genetic relatedness of cooperators (with respect to the dispersal genes they carry) within patches always increases the CS rate of dispersal ($dz^*/dr > 0$). This is because the indirect fitness benefits of dispersal, owing to the relaxation of resource competition for one's kin, scale with the relatedness of patch mates. When occupation deteriorates patches ($a \leq 1/2$), then increasing the proportion of habitable patches that are occupied (p) always decreases the CS rate of dispersal ($dz^*/dp < 0$). When occupation improves patches ($a > 1/2$), the CS rate of dispersal may be either an increasing or a decreasing function of the proportion of habitable patches that are occupied (p). This is because, even if occupation improves patches, dispersers may still be better off if they land on an unoccupied patch, as there are more individuals competing for resources on occupied patches. Hence, the direct fitness of a disperser may increase or decrease with the proportion of occupied patches (higher p), depending upon whether the improvement or competition effect dominates. In contrast, the indirect fitness of a disperser always decreases with increasing abundance of occupied patches (higher p), because it is increasingly likely that the freed up opportunities for reproduction in her natal patch will be won by immigrants rather than her nondispersing kin. Increasing the relative carrying capacity of occupied patches (a) always reduces the CS rate of dispersal ($dz^*/da < 0$). This is because any direct benefit of dispersal owes to the individual finding herself in a patch that is better than the one that she dispersed away from.

The relationship between the cost of dispersal (c) and the CS rate of dispersal (z^*) is qualitatively affected by both the proportion of habitable patches that are occupied (p) and relatedness (r). Increasing mortality cost of dispersal always reduces the direct fitness effect of dispersal and always increases the indirect fitness effect of dispersal. In Hamilton & May's (1977) classic model, the former effect always outweighs the latter, such that increasing mortality cost always reduces the CS rate of dispersal. However, this is not true in our more elaborate model. Consequently, whereas the CS rate of dispersal is

sometimes monotonically decreasing with increasing mortality cost for some parameter values, for others, it may be a U-shaped function of the mortality cost (see

also Gandon & Michalakis, 1999; Ronce, 2007). These results are summarized in Fig. 3.

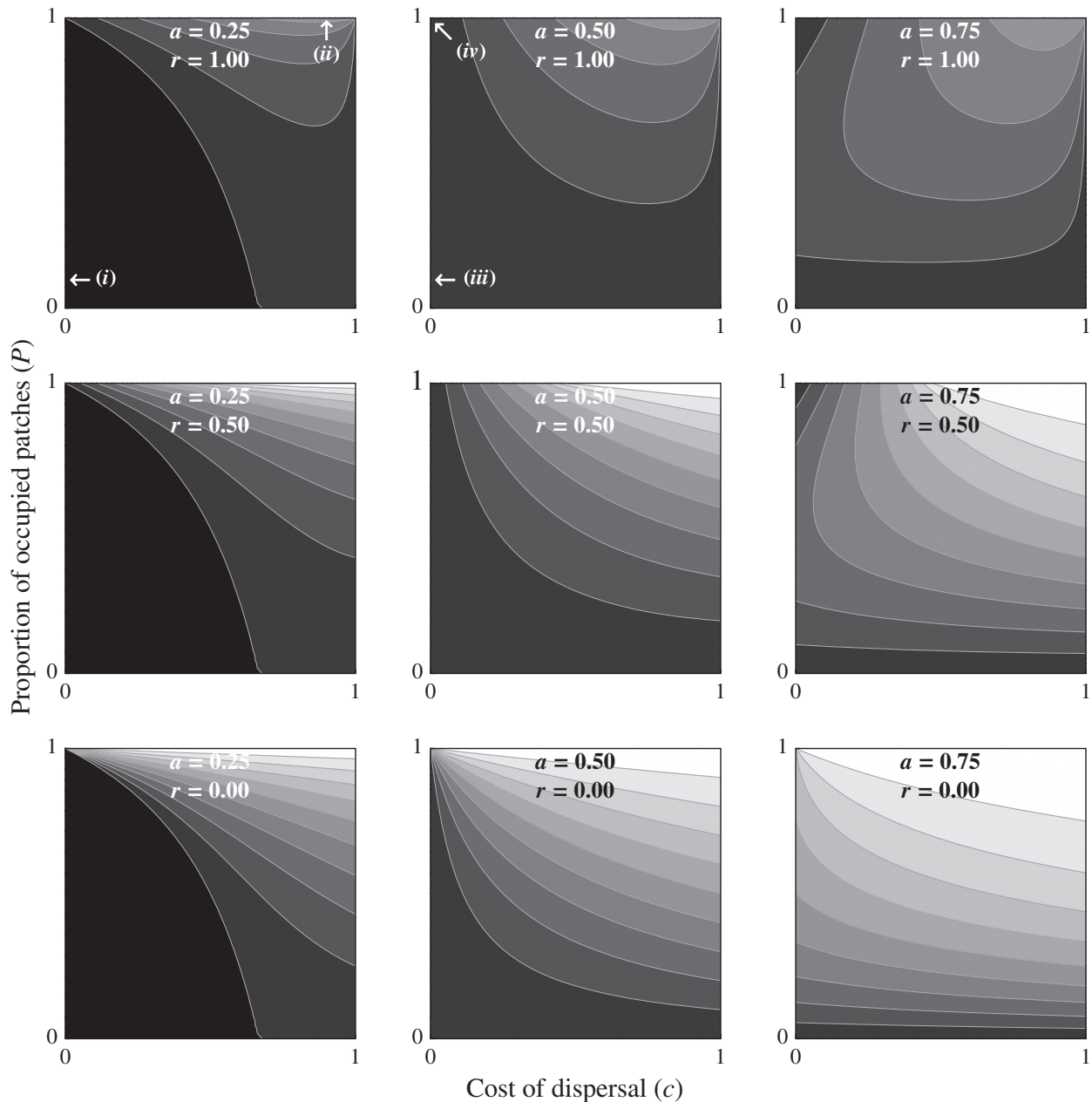


Fig. 3 Contour plots of the convergence stable (CS) rate of dispersal (z^*), as a function of the cost of dispersal (c ; abscissae), the proportion of habitable patches that are occupied (p ; ordinates), the relative carrying capacity of occupied patches (a ; columns) and the coefficient of genetic relatedness (r ; rows). The scale varies from $z^* = 0$ (no dispersal, white) to $z^* = 1$ (full dispersal, black). The CS rate of dispersal (z^*) decreases as the carrying capacity of the home patch (a) or the proportion of habitable patches that are occupied (p) increases, and decreases as the genetic relatedness within patches (r) increases. The relation between the CS rate of dispersal (z^*) and the cost of dispersal (c) is more complicated, and is mediated by relatedness (r). For relatively low relatedness ($r = 0.00, 0.50$), the CS rate of dispersal (z^*) decreases monotonically with the cost of dispersal (c). For relatively high relatedness ($r = 1.00$), the CS probability of dispersal (z^*) is a U-shaped function of the cost of dispersal (c). The arrows indicate the points in the parameter space that correspond to the four treatments of Fig. 1, hence: (i) low patch occupancy and cheats as social neighbourhood; (ii) high patch occupancy and cheats as social neighbourhood; (iii) low patch occupancy population and cooperators as social neighbourhood; (iv) high patch occupancy population and cooperators as social neighbourhood.

We now summarize the model results to qualitatively answer our main question: how does the presence of public goods cheats affect the evolution of dispersal under high patch occupancy and low patch occupation? Under low-occupancy conditions (β is high, α is low, and hence, p is low), dispersal will always evolve to be high (Fig. 2), because there are no costs of dispersal, only benefits, regardless of whether cheats are present (low a) or absent (high a) in occupied patches. Under high-occupancy conditions (β is low, and hence, p is high) in the absence of cheats (high a), dispersal also evolves to a high level, despite surrounding patches affording equal growth as the home patch, as a result of indirect fitness benefits: dispersal alleviates local kin competition (Hamilton & May, 1977). By contrast, high-occupancy conditions with a high frequency of cheat patches (high γ and low α , with $c < 1$) result in the evolution of lower dispersal, because the home patch where there are cooperators as well as cheats is a better environment than the surrounding pure cheat patches (Fig. 3).

Discussion

In this study, we investigated how patch occupation and social habitat heterogeneity affect the evolution of dispersal. We compared the relative cell densities of dispersing and nondispersing isogenic strains of the bacterium *P. aeruginosa* when cheats/cooperators were locally and globally present. We found that dispersal was always favoured when there were vacant patches available to colonize (low patch occupancy), but when patches were saturated (high patch occupancy), the presence of public goods cheats greatly altered selection for dispersal. Specifically, when public goods cheats were localized to the home patch, higher dispersal rates were favoured, but when cheats were present throughout available patches, lower dispersal rates were favoured. These results are qualitatively consistent with our theoretical model.

The results can be explained by the dispersing morph adopting a more risky strategy, depleting the numbers in the inoculation site and exposing itself to potential unknown costs within the environment (in this case, the cheats). This strategy pays off when the dispersers find themselves in high nutrient, virgin territory beyond the threat of the cheats (as in the low-occupancy treatment group), but dispersal is costly when there is a strong probability that cheats wait beyond the home site. On the other hand, the nondispersers will not expose themselves to external threats and will therefore dominate the home site – but not beyond – in all treatment groups. This was particularly apparent when cheats were at high patch occupancy: dispersers did not appear to grow beyond the range of the inoculation site, whereas nondispersers were able to slowly migrate beyond this area. Presumably, this is because the dispersers diminished their numbers in the inocula-

tion site as cells dispersed from the colonizing group, whereas the nondispersers maintained relatively high numbers in the inoculation site (because they are not losing cells to dispersal events). We can rule out the possibility that density dependence alone can explain the results given above due to the fact that dispersers reach a relatively higher density (i.e. are more fit) when cheat occupation is low and limited to the home patch. If the correlation between fitness and relatedness (at the dispersal loci) were entirely due to density dependence, dispersers should be even more fit when cooperators are locally confined to the home patch, because under these conditions, there will be a higher density of dispersing cooperators.

Our theoretical model also investigated the interaction between relatedness (with respect to dispersal strategy), the presence of public goods cheats and patch occupancy on the evolution of dispersal (Fig. 3). Relatedness was not manipulated in our experiment, experimental populations were isogenic, consisting of either dispersers or nondispersers, and this therefore corresponds with a theoretical scenario in which $r = 1$. However, by allowing relatedness to vary in our theoretical model, we were able to dissect the direct vs. indirect fitness mediators of the evolution of dispersal and, moreover, facilitate connections with the wider theoretical literature on the social evolution of dispersal. To understand this model effectively, it is important to clarify that although relatedness will often depend strongly on dispersal, it will not always. For example: if patches are founded by a single cell, there will be clonal relatedness within patches irrespective of the rate of dispersal; alternatively, budding dispersal can also allow for scenarios where dispersal is complete and there is clonal relatedness (Gandon & Michalakis, 1999). However, low relatedness generally means that direct benefits will drive the evolution of dispersal, such that patch occupancy determines the fitness of dispersing phenotypes: dispersal is favoured under low patch occupancy, regardless of the presence of cheats (as in the models of Van Valen, 1971; McPeck & Holt, 1992; Greenwood-Lee & Taylor, 2001; Leturque & Rousset, 2002). Under high relatedness, indirect benefits also drive the dispersal patterns, such that dispersal can be favoured under higher values of patch occupancy, because dispersal alleviates kin competition in the patch of origin (as in the models of Hamilton & May, 1977; Frank, 1986 and Gandon & Michalakis, 1999). Our model emphasizes the relative impact of differences in an individual's social environment and patch occupancy upon the evolution of dispersal. Our results are also in line with those of Le Galliard *et al.* (2003, 2005), who have analysed how altruism and mobility interact. They suggested that cooperators can either exhibit high mobility, owing to high local kin competition and relatively low cost of mobility, or exhibit low mobility, owing to high cost of mobility and relatively low local

kin competition. This is identical to our conclusions. However, whereas in their model, the cost of mobility is due to an environmental factor, and in our case, the analogous cost of dispersal is due to the presence of cheats in the environment.

Whereas the combination of theory and empirical work we present here helps us to both interpret and generalize the results from our simple experiment, it is important to emphasize the key limitations of our study. First, we did not allow dispersal phenotypes to evolve as a result of mutations generated *de novo* during the course of the experiment, but instead relied on measuring the fitness of defined mutants. Gene knockouts can be associated with large pleiotropic effects that can impact relative fitness between the strains. However, controls in shaken liquid and soft agar showed no significant difference in growth rate between the two strains when motility was unlikely to confer any advantage, and therefore, any fitness differences observed are likely the result of the dispersal phenotypes.

Second, a number of our theoretical model assumptions may limit generality, and alternative assumptions would change model predictions. We assumed that cheats do not disperse, whereas cheats within the experimental setting have intermediate dispersal. If cheats were dispersers, we would expect contrasting results. However, the key assumption in the model is that dispersers are able to escape cheats, whereas nondispersers cannot, and this was also the case in the experiments. This provides a mechanism for cooperators to escape cheat exploitation. Also, we defined relatedness only with respect to the dispersal strategy and not with respect to public good production, because our analysis concerns the evolution of the former rather than the latter trait. More complex models, which consider co-evolution of dispersal and cheating, would need to compute relatedness for both of these traits. However, using a combined empirical and theoretical approach clarifies and expands results from the empirical study alone. This allows the results to be understood in terms of direct and indirect fitness benefits that shape the evolution of dispersal by breaking down the factors of relatedness and patch quality.

Third, the social habitat in our experimental setup was continuous, whereas the model defines a patch-structured habitat. This limitation of the model allows greater analytical tractability and is more true to the experimental set-up. Lattice models differ from island models in that they take into account the geographic distance between subpopulations and individuals and might therefore provide a better approximation to a continuous habitat set-up (Rousset, 2004). However, previous studies of kin competition in genetically structured populations show that patch-structured populations (Taylor, 1992a) and lattice-structured populations (Taylor, 1992b) yield qualitatively similar predictions. Moreover, a comparative analysis of the evolution of

dispersal in a homogeneous population under different structures, ranging from patch-structured habitats to several variations of lattice-structured habitats, shows that although these different habitat structures give slightly different quantitative results, the qualitative results are similar (Gandon & Rousset, 1999). A particular major challenge for future studies, both theoretically and empirically, is to consider populations where habitat structure co-evolves with traits to bridge the gap between laboratory and natural settings (e.g. Jessup *et al.*, 2004; Johnson & Stinchcombe, 2007; Lion & van Baalen, 2008; Lehmann & Rousset, 2010).

Fourth, our theoretical results are given in terms of evolutionary endpoints, whereas the experimental results concern evolving populations that have not yet settled to equilibrium. This is a common limitation of experimental evolutionary studies (Buckling *et al.*, 2009; Kümmerli *et al.*, 2009b; Kawecki *et al.*, 2012). Indeed, this limitation is true of any application of comparative statics to biological populations. However, this approach remains one of the most successful in advancing our understanding of the selective forces underlying the adaptive evolution of organisms (Grafen, 1984, 1991; Frank, 1998 Ch 12; West, 2009).

Microbes engage in many collective actions, and this usually requires the maintenance of a kin-structured environment (Czárán & Hoekstra, 2009). For pathogens, maintaining social behaviours – many of which are important virulence factors (Rumbaugh *et al.*, 2009) – will also aid transmission by ensuring the host is inoculated with an infective dose (Hall-Stoodley & Stoodley, 2005). *Agrobacterium tumefaciens* is a plant pathogen that requires highly social groups to elicit a successful pathogenic attack on a host, and it has recently been proposed that for transmission to be effective, dispersal would have to occur via budding (Platt *et al.*, 2012). What is less well explored is the additional mechanism this behaviour might provide to escaping harmful invaders such as predators (Matz & Kjelleberg, 2005), toxin producers (Majeed *et al.*, 2011), parasites (Wilson & Sherman, 2010) or, indeed, cheats (Velicer, 2003).

The theoretical model offers generalization of our results beyond the microbial world. There has been much interest in investigating the role of kin competition and habitat saturation in driving the evolution of dispersal, and hence, there are many empirical examples that demonstrate increased dispersal driven by kin competition [such as in voles (Bollinger *et al.*, 1993) and insects (Kasuya, 2000)] and decreased dispersal driven by local patch occupancy [as seen in kangaroo rats (Jones, 1988), and black kites (Forero *et al.*, 2002)]. However, here, we are assuming not only the number of occupants, but also the nature of the occupants (cooperators or cheats), will influence the evolution of dispersal, and there is evidence that kin are often more favourable neighbours than nonkin. For example, many animals appear to show kin-biased habitat choice

[for example in the crow (Baglione *et al.*, 2003) and the lizard (Sinervo & Clobert, 2003)], and in addition, it has been shown that neighbour type (kin vs. nonkin) can also impose selection on important fitness traits. For example, in the Townsend's vole (*Microtus townsendii*), females tend to nest nearer to kin and those nesting in close proximity with kin have higher survival than those nesting near nonkin (Lambin & Krebs, 1993). Highly social organisms, such as the eusocial hymenoptera, can overcome conflicts between the benefits of dispersal and costs resulting from the breakdown of cooperation via budding dispersal – where groups from a larger colony will disperse together to ensure the founding colony will maintain high relatedness (Ross & Keller, 1995; Gardner & West, 2006; Kümmerli *et al.*, 2009b). Here, we suggest that dispersal can also provide a benefit to cooperative groups if it offers the opportunity to run-away from invading cheats; however, this risky strategy only pays off if population structure is such that escape is possible. A greater knowledge of the relative costs and benefits of associating with kin and nonkin helps to explain this result in more detail and adds to the body of work that describes mechanisms for the observed diversity of natural dispersal behaviours.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Data S1 Full details of theoretical model.

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